Published in final edited form as:

Bioorg Med Chem Lett. 2007 July 15; 17(14): 4026–4029.

Inhibition of IGF-1R and Lipoyxygenase by Nordihydroguaiaretic Acid (NDGA) Analogs

Joseph E. Blecha^a, Marc O. Anderson^a, Jennifer M. Chow^a, Christle C. Guevarra^a, Celia Pender^b, Cristina Penaranda^b, Marianna Zavodovskaya^b, Jack F. Youngren^b, and Clifford E. Berkman^a

^aDepartment of Chemistry & Biochemistry, San Francisco State University, San Francisco, CA 94132-4163

^bDiabetes and Endocrine Research, University of California, San Francisco/Mt. Zion Medical Center, Box 1616, San Francisco, CA. 94143 – 1616, USA

Abstract

Herein we pursue the hypothesis that the structure of nordihydroguaiaretic acid (NDGA) can be refined for selective potency against the insulin-like growth factor 1 receptor (IGF-1R) as a potential therapeutic target for breast cancer while diminishing its action against other cellular targets. Thus, a set of NDGA analogs (**7a-7h**) was prepared and examined for inhibitory potency against IGF-1R kinase and an alternative target, 15-lipoxygenase (15 LOX). The anti-cancer effects of these compounds were determined by their ability to inhibit IGF-1 mediated cell growth of MCF-7 breast cancer cells. The design of the analogs was based upon a cursory Topliss approach in which one of NDGA's aromatic rings was modified with various substituents. Structural modification of one of the two catechol rings of NDGA was found to have little effect upon the inhibitory potency against both kinase activity of the IGF-1R and IGF-1 mediated cell growth of MCF-7 cells. 15-LOX was found to be most sensitive to structural modifications of NDGA. From the limited series of NDGA analogs examined, the compound that exhibited the greatest selectivity for IGF-1 mediated growth compared to 15-LOX inhibition was a cyclic analog **7h** with a framework similar to a natural product isolated from *Larrea divaricata*. The results for **7h** are significant because while NDGA displays biological promiscuity, **7h** exhibits greater specificity towards the breast cancer target IGF-1R with that added benefit of possessing a 10-fold weaker potency against 15-LOX, an enzyme which has a purported tumor suppressing role in breast cancer. With increased specificity and potency, **7h** may serve as a new lead in developing novel therapeutic agents for breast cancer.

Nordihydroguaiaretic acid (NDGA, Figure 1) is isolated from the resinous extract of the creosote bush *Larrea divaricata*.¹ It has also been reported that NDGA inhibits the growth of tumors, both in vitro and in vivo² but relatively high concentrations are required to achieve efficacy in most cases. Preliminary in vivo studies have revealed that NDGA inhibits tumor growth by inhibiting receptor tyrosine kinase (RTK) phosphorylation.³ NDGA has been shown to inhibit several RTKs overexpressed in certain cancer cells. In a previous study, we noted that NDGA inhibits both the IGF-1R and HER2/neu with moderate potency and impairs subsequent signaling through the anti-apoptotic pathway in breast cancer cell.^{3, 4} These and other RTKs such as the epidermal growth factor receptor (EGFR) are overexpressed in breast and other cancers and contribute to a poor prognosis⁵⁻⁷.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

However, NDGA is known to directly interact with multiple non-RTK targets, several of which could contribute to its anti-cancer effects. NDGA directly inhibits the interaction of fatty acid synthase (FAS) with the substrate malonyl CoA⁸. NDGA can also directly inhibit 5- α reductase,⁹ thereby blocking the conversion of testosterone into the biologically active dihydrotestosterone. Inhibitors of both FAS and 5- α reductase can inhibit growth or induce apoptosis in certain cancer cell lines.^{9, 10} NDGA's reported antioxidant properties are attributed to its activity as a non-selective inhibitor of lipoxygenases (LOX).¹¹ The role of LOX isozymes in cancer has been questionable, as the anti-cancer effects of NDGA were originally attributed to reduction of cellular levels of leukotrienes generated from arachidonic acid by 5- and 12-LOX,¹²⁻¹⁴ but recent studies suggest that in breast cancer, 15-LOX has a tumor suppressing role.¹⁵ The multiplicity of NDGA's effects may, in some cases, enhance its anti-tumor actions, but may also antagonize its therapeutic effects or increase the undesirable side effects in non-tumor sites. Therefore, in designing potential therapeutics for breast cancer, compounds capable of inhibiting RTKs such as IGF-1R and HER2/neu but lacking potency against 15-LOX or other targets could prove promising. The development of NDGA analogs with more specific potencies for various cellular targets is expected to result in an enhancement of its anti-cancer properties.

The focus of the present study was to demonstrate that the structure of NDGA could be refined for selective potency against the IGF-1R as a therapeutic target for breast cancer¹⁶ while diminishing its action against 15-LOX. Primarily based upon a cursory Topliss approach,^{17, 18} our objective was to prepare a small set of NDGA analogs in which the substituents on one of NDGA's aromatic rings were adjusted to explore a range of hydrophobicity, electronics, and steric properties. We also pursued two simplified analogs of NDGA in which the methyl substituents on the bridging butyl chain and one of the hydroxyls of a catechol ring were absent.

The synthesis of NDGA analogs **7a-7g** is outlined in Scheme 1. Carboxylic acids **1a-1b** were initially reduced to the corresponding alcohols with LiAlH₄¹⁹ and subsequently oxidized to the intermediate aldehydes **2a-2b** with pyridinium chlorochromate (PCC).²⁰ Next, treatment with substituted-aryl Grignard reagents (**3a-3f**) yielded alcohols²¹ **4a-4g**, which were oxidized with PCC²⁰ to yield ketones **5a-5g** prior to their complete reduction.

As halogenated aryl rings can undergo dehalogenation under the simple conditions of catalytic hydrogenation to reduce benzylic ketones,²² the halogen-containing aryl ketones **5d-5e** were subjected to adapted Wolff-Kishner conditions employing hydrazine hydrate²³ to generate an initial hydrazone intermediate. Hydrazones were subsequently reduced with KOH^{23, 24} to yield alkanes **6d-6e**. The remaining non-halogen substituted aryl ketones (**5a-5c**, **5f**, and **5g**) were reduced by simple catalytic hydrogenation. Next, precursors **6a-6e** were demethylated with BBr₃²⁵ to yield the NDGA analogs **7a-7e**. 3,4-Methylenedioxy-protected catechol **6f** was selectively deprotected using PCl₅²⁶ to yield **7f**. Similar conditions were applied to **6g**, followed by subsequent demethylation with BBr₃ to generate **7g**, the desmethyl analog of NDGA. Both **7a** and **7g** have been reported previously.^{27, 28}

A cyclic NDGA analog **7h** was prepared as outlined in Scheme 2. Ketone **5a** was reduced with Et₃SiH²⁹ and TFA, and was expected to proceed through two steps of carbocation formation followed by reduction through hydrogen transfer by the silane. Applied to this system, these conditions generated product **9** which may have occurred through cyclization of the benzylic carbocation intermediate **8**. Subsequent demethylation provided cyclic NDGA analog **7h**. Incidentally, this cyclic framework has also been isolated as a plant-derived natural product in *Larrea spp.*³⁰

Compounds **7a-7h** were screened along with NDGA in three separate assays for activity. The first assay tested direct inhibition of IGF-1R phosphorylation of a synthetic, non-specific,

protein tyrosine kinase substrate. The second examined the inhibitory effect of the NDGA analogs on IGF-1 mediated cell growth of MCF-7 breast cancer cells. The third assay screened the analogs for the inhibition of 15-LOX, a non-target enzyme and one for which NDGA is a known inhibitor.³¹ The results from the three assays for NDGA and its synthetic analogs are summarized in Table 1.

In general, the analogs did not possess inhibitory potency against IGF-1R kinase activity distinct from NDGA. These results suggest that with respect to the structure of NDGA, a single catechol ring is sufficient to affect inhibition of IGF-1R. The inhibition of IGF-1 mediated growth of MCF-7 cells by NDGA and the analogs presented an approximate 3-fold range in potency. The slight variation of potency compared to the results of IGF-1R inhibition may be due to variances in passive membrane permeability, metabolism, or interactions with other cellular targets. In contrast, NDGA and its analogs displayed larger differences in their inhibitory potency against 15-LOX. The desmethyl analog (**7g**) exhibited similar anti-lipoxygenase activity as NDGA and both these compounds were the most potent in the series that were examined. Given the symmetry of NDGA and **7g**, the greater accessibility and probability of catechol rings binding in the enzyme may be responsible for their enhanced potency. Analogs possessing a single catechol ring and bearing sterically demanding substituents on the remote non-catechol ring exhibited weaker anti-lipoxygenase activity. The most dramatic loss of activity was observed for the cyclic analog **7h**, especially when directly compared to its linear congener **7a**. These results suggest that steric congestion near the catechol ring diminishes 15-lipoxygenase activity but has no effect upon the inhibition of IGF-1R and IGF-1R mediated cell growth of MCF-7 cells.

Significant structural modifications to one of the two catechol rings of NDGA had little effect upon the inhibitory potency against the kinase activity of the IGF-1R and only moderate differences in potency were observed when assayed for inhibition of IGF-1 mediated cell growth of MCF-7 cells. Of the three assays conducted, the inhibitory activity against 15-LOX was the most sensitive to structural modifications of NDGA. From the limited series of NDGA analogs examined, the compound that exhibited the greatest selectivity for IGF-1 mediated growth compared to 15-LOX inhibition was the cyclic analog **7h**. While the linear congener **7a** displayed similar potency as **7h** against IGF-1 mediated growth of MCF-7 cells it retained similar potency as NDGA against 15-LOX. These findings are significant because while NDGA displays biological promiscuity, **7h** exhibits greater specificity towards the breast cancer target IGF-1R with that added benefit of possessing a 10-fold weaker potency against 15-LOX, an enzyme which has a purported tumor suppressing role in breast cancer. With increased specificity and potency, **7h** may serve as a new lead in developing novel therapeutic agents for breast cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by a grant from the National Cancer Institute, U-56 CA92616. The authors would also like to extend their gratitude to W. Tam and the NMR facility at SFSU for their expert assistance.

References and Notes

1. Waller CW, Gisvold O. Journal of the American Pharmaceutical Association-Scientific Edition 1945;34:78.
2. Avis IM, Jett M, Boyle T, Vos MD, Moody T, Treston AM, Martinez A, Mulshine JL. J Clin Invest 1996;97:806. [PubMed: 8609238]

3. Youngren JF, Gable K, Penaranda C, Maddux BA, Zavodovskaya M, Lobo M, Campbell M, Kerner J, Goldfine ID. *Breast Cancer Res Treat* 2005;94:37. [PubMed: 16142439]
4. Domin J, Higgins T, Rozengurt E. *J Biol Chem* 1994;269:8260. [PubMed: 7510683]
5. Korc M, Chandrasekar B, Yamanaka Y, Friess H, Buchler M, Beger HG. *Journal of Clinical Investigation* 1992;90:1352. [PubMed: 1401070]
6. Bergmann U, Funatomi H, Yokoyama M, Beger HG, Korc M. *Cancer Res* 1995;55:2007. [PubMed: 7743492]
7. Yamanaka Y, Friess H, Kobrin MS, Buchler M, Kunz J, Beger HG, Korc M. *Hum Pathol* 1993;24:1127. [PubMed: 8104858]
8. Li BH, Ma XF, Wang Y, Tian WX. *J Biochem (Tokyo)* 2005;138:679. [PubMed: 16428296]
9. Maria MA, Morrissey C, O'Keane C, Mulligan N, Watson C, Smith J, Fitzpatrick JM, Watson RW. *Cancer* 2006;106:2743. [PubMed: 16703599]
10. De Schrijver E, Brusselmans K, Heyns W, Verhoeven G, Swinnen JV. *Cancer Res* 2003;63:3799. [PubMed: 12839976]
11. Van Wauwe J, Goossens J. *Prostaglandins* 1983;26:725. [PubMed: 6422509]
12. Chang J, Skowronek MD, Cherney ML, Lewis AJ. *Inflammation* 1984;8:143. [PubMed: 6430801]
13. Cooke RG, Ahmad N. *J Endocrinol* 1994;142:47. [PubMed: 7964283]
14. Miyazawa K, Iimori Y, Makino M, Mikami T, Miyasaka K. *Jpn J Pharmacol* 1985;38:199. [PubMed: 3928952]
15. Jiang WG, Watkins G, Douglas-Jones A, Mansel RE. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 2006;74:235.
16. Kucab JE, Dunn SE. *Breast Dis* 2003;17:41. [PubMed: 15687676]
17. Topliss JG. *J Med Chem* 1977;20:463. [PubMed: 321782]
18. Topliss JG. *J Med Chem* 1972;15:1006. [PubMed: 5069767]
19. McDonald RW, Bunjobpon W, Liu T, Fessler S, Pardo OE, Freer IK, Glaser M, Seckl MJ, Robins DJ. *Anticancer Drug Des* 2001;16:261. [PubMed: 12375879]
20. O'Neill JA, Gallagher OP, Devine KJ, Jones PW, Maguire AR. *J Nat Prod* 2005;68:125. [PubMed: 15679335]
21. Monte WT, Lindbeck AC. *Org Proc Res Devel* 2001;5:267.
22. Pavlenko NV. *Uspekhi Khimii* 1989;58:767.
23. Brana MF, Cacho M, Garcia ML, Mayoral EP, Lopez B, de Pascual-Teresa B, Ramos A, Acero N, Llinares F, Munoz-Mingarro D, Lozach O, Meijer L. *J Med Chem* 2005;48:6843. [PubMed: 16250643]
24. Cram DJ, Sahyun MRV, Knox GR. *J Am Chem Soc* 1962;84:1734.
25. Vickery EH, Pahler LF, Eisenbraun EJ. *J Org Chem* 1979;44:4444.
26. Koelsch CF. *J Am Chem Soc* 1946;68:148.
27. Howell, S.; Khandwala, A.; Sachdev, OP.; Smith, CG. US Patent. 5,541,232. 1996.
28. McDonald RW, Bunjobpon W, Liu T, Fessler S, Pardo OE, Freer IKA, Glaser M, Seckl MJ, Robins DJ. *Anti-Cancer Drug Design* 2001;16:261. [PubMed: 12375879]
29. West CT, Donnelly SJ, Kooistra DA, Doyle MP. *J Org Chem* 1973;38:2675.
30. Gnabre J, Huang RCC, Bates RB, Burns JJ, Caldera S, Malcomson ME, McClure KJ. *Tetrahedron* 1995;51:12203.
31. Whitman S, Gezinci M, Timmermann BN, Holman TR. *J Med Chem* 2002;45:2659. [PubMed: 12036375]

Figure 1.
Structure of nordihydroguaiaretic acid (NDGA).

Scheme 1.

Reagents and conditions: a) LiAlH_4 , CH_2Cl_2 , RT, 3.5h b). PCC, CH_2Cl_2 , RT, 1h c) Et_2O or THF, -78°C , then RT, 30 min. d) PCC, CH_2Cl_2 , RT, 1h. e) H_2 , 10% Pd/C, THF, RT, 1.5h f) $\text{H}_2\text{NNH}_2\cdot\text{H}_2\text{O}$ and KOH, 165°C , 4h. g) BBr_3 , CH_2Cl_2 , -78°C , then RT, 30 min. h) PCl_5 , PhMe, 160°C , then H_2O .

Scheme 2.

Reagents and conditions: a) TFA, Et₃SiH, 0 °C then RT, 2.5h. b) BBr₃, CH₂Cl₂, -78 °C, then RT 30 min.

Table 1
Inhibitory Potency and Selectivity of NDGA analogs 6a-6h.^a

Inhibitor	IGF-1R Inhibition IC ₅₀ (μM)	Inhibition of IGF-1 mediated growth IC ₅₀ (μM)	15-Lipoxygenase Inhibition IC ₅₀ (μM)
NDGA	0.9 (0.3)	24.6 (10.7)	3.8 (0.8)
7a	0.8 (0.1)	11.2 (1.8)	7.3 (0.9)
7b	0.8 (0.1)	15.1 (4.1)	21.6 (0.5)
7c	1.0 (0.1)	36.2 (6.4)	9.4 (0.3)
7d	0.8 (0.4)	13.4 (3.3)	15.0 (5.5)
7e	1.0 (0.2)	21.5 (3.2)	33.6 (1.6)
7f	1.1 (0.1)	15.7 (4.0)	15.0 (1.4)
7g	0.6 (0.1)	25.4 (6.6)	3.9 (1.1)
7h	1.0 (0.4)	12.3 (2.4)	38.6 (1.7)

^aIC₅₀ values represent the mean of a minimum of 3 determinations with standard deviation in parenthesis.